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TEMPERATURE AS A FACTOR IN THE DETERMINATION OF SEX IN AMPHIBIANS.

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In 1905, Hertwig (1) published the results of a series of experiments on the eggs of *Rana temporaria* and of *Rana esculenta* which showed, according to his interpretation, that temperature is a sex-determining factor in amphibians: a high temperature favoring the development of females; a low temperature causing the production of relatively more males. This conclusion is in accord with the results of Issakówitsch's (2) earlier experiments on sex-determination in *Daphnia*; but it is opposed by the results of Maupas' (7) experiments on *Hydatina senta* and by those of von Malsen (6) on *Dinophilus apatris*. The latter experiments seemed to indicate that heat leads to the development of males and that cold tends to the production of females. Maupas and Hertwig believe that temperature acts directly on the sex-cells: Issakówitsch and von Malsen, on the other hand, maintain that nutrition is the dominant factor in sex-determination, temperature acting only indirectly through its influence on the processes of assimilation in the parent organism.

In the spring of 1907, I made a series of temperature experiments on the eggs of the toad, *Bufo lentiginosus*, which were carried out in a manner similar to those made by Hertwig on the eggs of *Rana*. Various lots of fertilized eggs were placed in tanks in which the water was kept at a nearly constant temperature until the tadpoles underwent metamorphosis (King, 5). In *Bufo* as in *Rana*, the sexes cannot be distinguished until the end of metamorphosis unless the gonads are sectioned. It is conceivable, therefore, that sex in these forms may not be determined until a relatively late period and that temperature, acting during the entire growth period of the tadpoles, might alter the normal sex-ratio. Two series of experiments were made with eggs from different females. In one series (lot A) 62.5 per cent. of the in-

dividuals in which sex was ascertained were females, although one half of the eggs had developed in water which had a temperature of 22–30° C. while the other half of the eggs had been kept at a temperature of 14–18° C. In the second series (lot B), the eggs were subjected to conditions similar to those under which the eggs of lot A had developed. Only 33.97 per cent. of females was obtained in this series, and more females were found among the individuals which had developed at a temperature of 14–18° C. than among those which had been kept at the higher temperature. The results of these experiments, therefore, do not support Hertwig's contention, and they seem to indicate that temperature, acting during the development of the tadpoles, does not determine sex in *Bufo*.

In the series of experiments described above the eggs belonging to lot A were fertilized in water which had a temperature of 16–18° C.; the eggs used for lot B, on the other hand, were fertilized in water which had a temperature of only 11–13° C. The results obtained in the experiments suggested the idea that temperature, acting at the time that the eggs were fertilized, might determine sex. This suggestion was tested in the spring of 1908 in the following way: a pair of toads were placed in water which was kept at a temperature of 26° C. until the eggs had been deposited and fertilized; the eggs from a second female were laid and normally fertilized in water which had a temperature of 9° C. There was thus a difference of 17° C. in the temperature of the water in which these two sets of eggs were fertilized. In each series as many individuals as possible were carried through to metamorphosis and their sex ascertained. The results obtained in these experiments are summarized in the following table.

TABLE I.

Water Temperature.	Number Sex Ascertained.	Males.	Females.	Per Cent. Females.
26° C.	2,178	953	1,225	56.24
9° C.	2,083	1,181	902	43.30

The average proportion of females among young toads that have recently completed their metamorphosis is 51.62 per cent., judging from the number of females found among the 9,949

individuals whose sex I have ascertained during the course of the experiments which I have made to study the problem of sex-determination in this form. No very definite conclusions can be drawn from the results of the experiments summarized in Table I. The percentage of females obtained from the lot of eggs that was fertilized at a temperature of 26°C ., although higher than the probable average for the species, is still within the limits of possible normal variation in the proportion of females developing from eggs laid by different individuals. A relatively low percentage of females developed from the lot of eggs that was fertilized at 9°C ., yet this percentage is about the same as that obtained in one of the series of experiments made to ascertain the influence of nutrition on the determination of sex in *Bufo* (King, 4). It may be possible to explain the results obtained in this last series of experiments, as well as those of the first series of temperature experiments briefly described above, as due to the fact that occasionally a batch of eggs gives an unusually low or an unusually high percentage of females and that by chance such exceptional lots of eggs were used for these experiments.

As this last series of experiments was carried out it is open to serious criticism. The eggs that were fertilized in warm water were laid by one female, while those that were fertilized in the cold water were laid by another individual. This gives an opportunity for the results to be influenced by possible normal variations in the sex-ratios of different lots of eggs. The two sets of eggs were fertilized by sperm from different males also and, as Morgan (8) has pointed out, there is the possibility that the male is responsible for the determination of sex in amphibians.

In order to avoid all obvious sources of error and, if possible, to obtain results that would give definite conclusions, another series of experiments was made this past spring with the eggs of *Bufo*. On April 7, 1909, a female which had just begun to lay was killed by pithing and the eggs removed to a dish of fresh water. Lots of about 350 eggs each were artificially fertilized in water which was kept at given temperatures (35°C ., 30°C ., 20°C ., 10°C ., 5°C .) for twenty minutes and then allowed to come gradually to the room temperature (18°C .). In all cases sperm from the same male was used. The extreme temperatures at

which lots of eggs were fertilized were 35° C. and 5° C. It is highly improbable that in a state of nature the eggs of *Bufo* are ever fertilized at these temperatures which are very injurious to the unsegmented egg, although they do not seriously interfere with the development of segmented eggs unless allowed to act for a comparatively long period of time (King, 3). Not more than one half of the eggs subjected to extreme temperatures were fertilized. Segmentation of these eggs did not begin until some three hours after the experiments were started, and in a majority of cases the cleavage planes came in very irregularly. A considerable number of eggs that began segmentation died within a few hours, so that but a very small number of individuals continued their development. After the eggs in these lots had reached the gastrulation stage they had apparently outlived the injurious effects of extreme temperature, as only three of the tadpoles that developed from the eggs that were fertilized in water with a temperature of 35° C. and but two from those that were fertilized at 5° C. died before it was possible to ascertain their sex. The great majority of eggs that were fertilized at medium temperatures, 30° C., 20° C. and 10° C., segmented normally and continued their development. The mortality in these lots was very low, not more than 6–8 per cent. of the individuals dying before it was possible to ascertain their sex. The various lots of tadpoles received similar food and were all kept as nearly as possible under similar external conditions during their entire growth period.

In this series of experiments, and also in several earlier ones, many of the tadpoles died after beginning their metamorphosis. In a considerable number of these individuals it was necessary to section the gonads in order that sex might be ascertained. The toads that survived metamorphosis were placed in large glass jars containing moist sod, and they were fed on small insects for several days. Young toads grow very rapidly, and by keeping them in the manner indicated for a short time it is possible to ascertain the sex of practically every individual without resorting to the tedious process of preserving and sectioning the gonads.

The results of this series of experiments are summarized in Table II.

TABLE II.

Water Temperature.	Number Sex Ascertained.	Males.	Females.	Per Cent. Females.
35° C.	90	42	48	53.33
30° C.	197	96	102	51.26
20° C.	323	157	166	51.39
10° C.	214	108	106	49.53
5° C.	64	33	31	48.43
Total	888	436	451	50.90

With a difference of 30° C. in the temperature of the water in which the two lots of eggs at the extremes of the series were fertilized there is a difference of only 4.95 per cent. in the proportion of females that developed in these lots. This difference is so small that it is evident that temperature, acting at the time of the fertilization of the eggs, is not the dominant factor in the determination of sex in *Bufo*.

In this series of experiments, with one exception, the proportion of females that was obtained in the different lots varied directly as the temperature at which the eggs were fertilized; relatively fewer females developing as the temperature of the water was lowered. The decrease in the percentage of females was so slight in the various cases, however, that I do not think it can have much significance, particularly as there were so few individuals in the lots at the extremes of the series. Had the number of individuals that metamorphosed been the same in every lot, practically the same sex-ratio would doubtless have been obtained for the entire series.

For the purpose of comparison, and to show more conclusively than is shown by the results of the series of experiments summarized in Table II. that temperature, acting at the time of the fertilization of the egg, does not determine sex in *Bufo*, the results obtained in former experiments in which different batches of eggs were laid in water with a known temperature are brought together in Table III.

TABLE III.

Water Temperature.	Number Sex Ascertained.	Males.	Females.	Per Cent. Females.
26° C.	2,178	953	1,125	56.24
16-18° C.	232	87	145	62.50
11-13° C.	156	103	53	33.97
9° C.	2,083	1,181	902	43.30

A comparison of the figures given in Table II. with those of Table III. shows that there is not the uniformity in the results of these various series of experiments that one would expect to find if temperature alone determined sex at the time of the fertilization of the egg. A lot of eggs that was fertilized at a temperature of 26° C. gave a higher percentage of females than was obtained from a lot that was fertilized at a temperature of 35° C., while the highest percentage of females obtained as yet in any experiment (62.5 per cent.) was found in a lot of individuals that developed from eggs that were fertilized at a temperature of 16–18° C. Of the various series of eggs that were fertilized in cold water, those that were fertilized at 9° C. gave a much lower percentage of females than did other lots that were fertilized at 10° C. or at 5° C. Although these results show very conclusively that temperature, acting at the time of the fertilization of the egg, is not the dominant factor in the determination of sex in *Bufo*, they do not exclude the possibility that temperature may have an indirect action on the determination of sex in this form. In every series of experiments lots of eggs that were fertilized at a temperature of 13° C. or below have given a lower percentage of females than has been obtained in the individuals developing from lots of eggs that were fertilized at higher temperatures. It is conceivable that a low temperature might act more injuriously on the female-producing spermatozoa than on those that are male-producing, if it be that there is a dimorphism in the spermatozoa of *Bufo* and that the male determines sex. This would, of course, greatly increase the chances that an egg laid in cold water would be fertilized by a male-producing spermatozoön. If, on the other hand, sex is determined in the egg, it is possible that the sex-determining mechanism is so evenly adjusted that temperature, under certain conditions, may turn the scale in one direction or the other.

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